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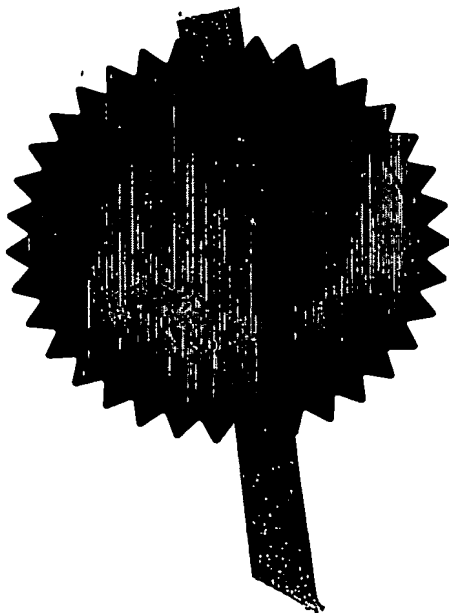
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Stephen Hordley

Dated

3 September 2004

- 5 AUG 2003

1/77

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1. Your reference RVCW / P28211GB 05AUG03 E827729-1 002866

2. Patent application number (The Patent Office will fill in this part) 0318247.4 P01/7700 0.00-0318247.4

3. Full name, address and postcode of the or of THE Royal Veterinary College (University of London) 5 AUG 2003

(Royal College Street
London NW1 0TU
United Kingdom)

Patents ADP number (if you know it)

8504177001

If the applicant is a corporate body, give the country/state of its incorporation

United Kingdom

4. Title of the invention ANTIGEN DELIVERY SYSTEM

5. Name of your agent (if you have one) ERIC POTTER CLARKSON

"Address for service" in the United Kingdom to which all correspondence should be sent (including the postcode)

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1305010

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Country

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Description 32

Claims(s) 7

Abstract 1

Drawing(s) 7 + 3

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Priority Documents 0

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Statement of inventorship and right to grant of a patent (*Patents Form 7/77*) NO

Request for preliminary examination and search (*Patents Form 9/77*) NO

Request for substantive examination (*Patents Form 10/77*) NO

Any other documents
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11. I/We request the grant of a patent on the basis of this application.

Eric Potter

Signature
ERIC POTTER CLARKSON

Date
4 August 2003

12. Name and daytime telephone number of person to contact in the United Kingdom 0115 9552211

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ANTIGEN DELIVERY SYSTEM

The present invention relates to an antigen delivery system. In particular, it relates to a system that can be used to vaccinate animals against diseases
5 and to discriminate between vaccinated and naturally infected animals.

Understanding and selective manipulation of the natural innate and adaptive immune system in animals is highly favourable both from a view of protecting animals, both farm animals as well as companion animals, from
10 disease and from a decrease in reliance on antibiotic intervention for disease control due to the increasing risks of antibiotic resistance, a topic of increasing importance in all species.

Antibiotics are frequently utilised on an indiscriminate basis for protection
15 of agricultural and companion animal species against a wide range of pathogens. This culture of inappropriate antibiotics use has developed through poor performance of pathogen antigens with respect to protective immunity.

20 Therefore, the need to develop and improve our understanding of the role of host adaptive immunity in pathogen protection is being driven, for example, both by animal welfare concerns and the increasing requirements of consumers for high quality meat with reduced antibiotic residues.

25 There is now an increasing research on molecular antigens with the aim of inducing protection in animal species by their use in vaccine products. However, because such products rarely take into account the requirements for priming of the cellular immune system with regard to protection of animals from disease, these have had limited efficacy in many cases.

One of the limitations of vaccination as a means of disease control is the difficulty of distinguishing naturally infected animals and vaccinated animals. For example, this was a major issue in relation to the recent foot and mouth disease (FMD) outbreak in the UK, where vaccination was not
5 used partly for the reason that once a national cow herd is vaccinated, certain countries will not allow imports of cattle from the herd. Present vaccination methods also lowers the market value of the meat as the carcass has to be treated as if it is infected, and left to hang for longer to kill any virus that may be present. For FMD the emphasis is on keeping clean
10 herds, but it is almost impossible to keep clean herds as transmission is a problem (eg from rats, badgers etc). Marker vaccines, which contain an immunogen foreign to the vaccinated animal, have been proposed as a way of distinguishing naturally-infected and vaccinated animals.

15 I propose that targeting dendritic cells using a moiety which selectively binds to a dendritic cell, in combination with an antigen such as one relevant to an animal disease, and which moiety which selectively binds as said is not naturally occurring in that animal species, provides not only a new concept of antigen delivery (vaccination), but also allows the
20 discrimination between vaccinated and naturally infected animals.

A first aspect of the invention provides a compound for vaccination of an animal comprising (i) a moiety which selectively binds to a dendritic cell in the animal but which moiety does not naturally occur in the animal and (ii)
25 an antigen.

By "moiety which selectively binds to a dendritic cell" I mean any suitable such moiety which binds a dendritic cell but does not substantially bind to other types of antigen presenting cells. Whether a moiety selectively binds
30 as said can be determined by measuring binding of the moiety to dendritic

cells compared to binding to other antigen presenting cells, for example using fluorescently labelled binding moiety and measuring the fluorescence associated with dendritic cells and other antigen presenting cells. Alternatively, an anti-moiety antibody can be used to determine which cells
5 the moiety binds to in order to determine its selectivity for dendritic cells.

Dendritic cells are highly potent antigen presenting cells and have been shown to be effective as a physiological adjuvant for eliciting prophylactic or therapeutic immunity. They capture microorganisms that enter
10 peripheral mucosal tissues and then migrate to secondary lymphoid organs, where they present these micro-organisms in antigenic form to resting T cells and thus initiate adaptive immune responses.

The moiety which selectively binds to a dendritic cell may be any suitable moiety. In particular, it is known that dendritic cells have present on their
15 surface proteins, typically receptors, which are selectively expressed and which may be used as a target for a dendritic cell. Thus, conveniently, the moiety binds to a receptor which itself is selectively expressed on dendritic cells. Typically, a receptor which is selectively expressed on a dendritic
20 cell is one which is present at a level 10-fold, preferably 100-fold, or more preferably 1000-fold higher on dendritic cells than other antigen presenting cells.

Preferably the moiety binds to a pattern recognition receptor on a dendritic
25 cell. A pattern recognition receptor is a receptor that binds a pathogen associated molecular pattern. Typically, a pattern recognition receptor is one which binds a lectin. Suitable pattern recognition receptors include DC-SIGN, DEC-205 (see, for example, Steinman *et al* (1997) *Immunol. Rev.* **156**, 25-37) and TOLL-like receptor (see, for example, Werling &
30 Jungi (2003) *Vet. Immunol. Immunopathol.* **91**, 1-12). More preferably, the

moiety binds to the cell-specific intercellular adhesion molecule 3-grabbing nonintegrin C-type lectin, DC-SIGN, which is highly expressed on the surface of dendritic cells. DC-SIGN is also sometimes called CD209.

- 5 DC-SIGN is a type II transmembrane protein which forms an N-terminal cytoplasmic domain, a transmembrane domain, a neck region, a tandem repeat region and a C-type (calcium dependent) mannose-binding lectin domain on its C-terminus. In human and mouse DC-SIGN is expressed by dendritic cells present in the dermis, lamina propria of mucosal tissues such
10 as rectum, uterus and cervix, and in the T-cell area of tonsils, lymph nodes and spleen. In humans DC-SIGN has 404 amino acids.

DC-SIGN has been found in human, chimp, gorilla, macaca, and where the cDNA encoding the polypeptide has been cloned. The cDNA and amino
15 acid sequences for DC-SIGN are found in the following GenBank Accession Nos AF391086 (*Macaca mulatta*), AY078913 (*Pan troglodytes*), NM_021155 (Human) and NM_133238 (*Mus*). Figure 1 shows a partial cDNA sequence of bovine DC-SIGN and the deduced amino acid sequence, aligned with DC-SIGNs from chimp, macaca and human, is shown in
20 Figure 3 (see Example 7 for details of the cDNA cloning). It is believed to be selectively expressed on the surface of dendritic cells and because of its role in the immune system is believed to be conserved between species.

Thus, for example, the cDNA encoding the amino acid sequences of human
25 and mouse DC-SIGN share 44% sequence similarity when assessed by the computer program described in the figure legends.

Thus, by DC-SIGN I include any protein whose cDNA encoding the polypeptide sequence has at least 40% similarity with the human or mouse
30 amino acid sequence shown in Figure 2.

In addition, the DC-SIGN molecule is typically one which is able to bind ICAM-3 or other members of the ICAM family as can be determined by, for example, by assays described in Geijtenbeek *et al* (2002) *J. Biol. Chem.* 277, 11314-11320.

The following articles describe the interaction between DC-SIGN and HIV-1 or ICAM-3, and are all incorporated herein by reference: Geijtenbeek *et al* (2002) *J. Leukoc. Biol.* 71, 921-931; Geijtenbeek *et al* (2002) *J. Biol. Chem.* 277, 11314-11320; Geijtenbeek *et al* (2000) *Cell* 100, 575-585; and Kooyk & Geijtenbeek (2002) *Immunol. Rev.* 186, 47-56.

In addition, I include any protein whose polypeptide is encoded by a polynucleotide which hybridises under stringent conditions to the polynucleotide whose sequence is given in any one of GenBank Accession Nos AF391086, AY078913, NM_021155 or NM_133238.

By "stringent conditions" I mean hybridising at 6xSSC and 60°C for at least 1 hour, and subsequent washing in 2xSSC at 60°C for 30 minutes. 1xSSC is 0.15M NaCl/0.015M sodium citrate.

Also I include any protein which is immunologically cross-reactive with human or mouse DC-SIGN. Typically, this may be assessed using polyclonal antisera to human or mouse DC-SIGN.

Preferably the moiety that selectively binds to a dendritic cell is a protein.

The compound is typically used for vaccination of an animal. Typically, the moiety that selectively binds is all or part of a molecule exogenous or foreign to the animal. It may be from another animal, such as man, and is

one which is immunogenic in, and gives rise to an antibody response in, the animal. Thus, it will be seen that not only does the moiety that selectively binds as said target the antigen to the dendritic cell, it will itself also give rise to an immune (antibody) response which serves as a marker of vaccination. In addition, the moiety which selectively binds may also act in stimulating the immune system, typically by priming a Th1 response, and so acts as an adjuvant.

Various moieties have been shown to bind DC-SIGN including the LAM protein of *Mycobacterium tuberculosis* (see, for example, Tailleux *et al* (2003) *J. Exp. Med.* **197**, 1-5), a glycoprotein of Ebola virus and the HIV-1 envelope glycoprotein gp 120 (see, for example, Geijtenbeek *et al* (2002) *J. Biol. Chem.* **277**, 11314-11320). It is preferred if the binding moiety is able to bind to DC-SIGN with high affinity. It will be appreciated that only a portion of these molecules are required to effect binding to a dendritic cell. Such portions are included in the term "a moiety which binds a dendritic cell". It is preferred, however, if all or substantially all of the molecule is used as the binding moiety.

It is particularly preferred if the moiety that selectively binds to a dendritic cell is HIV-1 envelope glycoprotein gp 120. Under natural occurring infections, DC-SIGN mediates HIV transfer by dendritic cells from mucosal surfaces (site of exposure to HIV) to secondary lymphoid organs (site of infection by HIV). This transfer can take several days during which HIV is protected from degradation and retains its infectivity. One potential mechanism is through endocytosis of HIV upon binding DC-SIGN only to return to the cell surface after arrival of the dendritic cell at the lymph node and interaction with T-cells. This internalization hypothesis is supported by the presence of two potential endocytosis motifs, LL and YXXL, in the cytoplasmic domain of DC-SIGN. These motifs have been shown to

mediate endocytosis and recycling in various contexts. HIV-1 bound to DC-SIGN transiently expressed in 293T-cells retains its infectivity for several days but is susceptible to trypsin treatment. This may point to the inability of DC-SIGN-expressing 293T-cells to endocytose HIV-1.

5 However, it is possible for HIV-1 internalization to be a significant mechanism of HIV-1 protection in dendritic cells.

The LL and YXXL motifs are conserved between species, as the homologue of the human DC-SIGN molecule has been found in mouse to contain these
10 motifs.

As the initial binding of HIV-1 is mediated by its gp 120 envelope protein to DC-SIGN expressed on dendritic cells and given the importance of DC-SIGN in HIV uptake, transport and transmission to T-cells, antigens linked
15 to the gp 120 molecule may be used to directly target dendritic cells with a very high efficiency, thus providing an example of the new vaccination approaches described herein.

Thus, targeting DC-SIGN using HIV gp 120 protein would: (a) stimulate
20 the host innate immune response by targeting the most potent antigen presenting cells and thus potentially limiting the number of application of the vaccine, the amount needed per vaccination, as well as eliminating the fear of either antibiotic residues or the outgrow of resistant bacterial strains; and (b) enabling the discrimination of vaccinated and naturally infected
25 animals by analysing the antibody response to the HIV gp 120 molecule, a protein not naturally occurring in the vaccinated animal, using commercially available test systems.

The invention also includes, specifically, a compound for vaccination of an
30 animal comprising (i) any of HIV gp120, the LAM protein of

Mycobacterium tuberculosis or a glycoprotein of Ebola virus, or parts thereof, and (ii) an antigen.

Preferably, the parts thereof are able to bind to a dendritic cell, typically to
5 DC-SIGN. Typically, the part thereof is greater than 10 amino acid residues in length, more typically greater than 15, 20, 25, 30, 40, 50, 60 or 70 amino acid residues in length.

Although the mycobacterial LAM protein binds DC-SIGN and so may be
10 useful in the practice of the invention, it will be appreciated that some free-ranging animals such as cattle, sheep, horses, goats, deer and so on are in contact with mycobacteria and so may be infected with them. Accordingly, it is preferred that the mycobacterial LAM protein is not used as the moiety which binds to a dendritic cell when the animal is a free ranging animal (or
15 other animal which may be infected with mycobacterium).

Preferably, the compound is one which is endocytosed by the dendritic cell following binding to said cell.

20 By "antigen" I include any moiety which can elicit an immune response, whether humoral or cell mediated, for example through the production of antibodies, and CD8-mediated, and NK cell-mediated responses. The antigen may refer to an individual molecule or to a homogeneous or heterogeneous population of antigenic molecules. Various macromolecules
25 can act as antigens, including all proteins (if present in the correct context), including nucleoproteins, lipoproteins, most polysaccharides (especially large polysaccharides), and various small molecules (usually called haptens) if they are attached to proteins or polypeptides or other carriers. The term "antigen" also includes antigenic molecules that are multivalent, having
30 multiple epitopes, or monovalent, having only one epitope.

In the context of vaccine production, the antigen is a molecule, or part thereof, or variant thereof, associated with a disease. In particular, in the context of an animal vaccine the antigen is a molecule, or part thereof, associated with a disease of the animal to be vaccinated. Antigens are associated with diseases involving a pathogen, particularly infectious diseases, and it is particularly preferred if the antigen is an antigenic or immunogenic portion of a component of a pathogen, such as an infectious microorganism.

10

Pathogens include those associated with the following diseases: foot and mouth disease, swine vesicular disease, peste des petits ruminants, lumpy skin disease, bluetongue, African horse sickness, classical swine fever, Newcastle disease, vesicular stomatitis, rinderpest, contagious bovine pleuropneumonia, Rift Valley fever, sheep pox and goat pox, African swine fever, highly pathogenic avian influenza. These are transmissible diseases that have the potential for very serious and rapid spread, irrespective of national borders, that are of serious socio-economic or public health consequence and that are of major importance in the international trade of animals and animal products and are List A diseases of OIE (Office International des Epizooties; www.oie.int).

20

Pathogens also include those associated with the following: Multiple species diseases including anthrax, Aujeszky's disease, echinococcosis/hydatidosis, heartwater, leptospirosis, new world screwworm (*Cochliomyia hominivorax*), old world screwworm (*Chrysomya bezziana*), paratuberculosis, Q fever, rabies, trichinellosis; cattle diseases including bovine anaplasmosis, bovine babesiosis, bovine brucellosis, bovine cysticercosis, bovine genital campylobacteriosis, bovine spongiform encephalopathy, bovine tuberculosis, dermatophilosis, enzootic bovine

30

- leukosis, haemorrhagic septicaemia, infectious bovine rhinotracheitis/infectious pustular vulvovaginitis, malignant catarrhal fever, theileriosis, trichomonosis, trypanosomosis (tsetse-borne); sheep and goat diseases including caprine and ovine brucellosis (excluding *B. ovis*), caprine
- 5 arthritis/encephalitis, contagious agalactia, contagious caprine pleuropneumonia, enzootic abortion of ewes (ovine chlamydiosis), maedi-visna, Nairobi sheep disease, ovine epididymitis (*Brucella ovis*), ovine pulmonary adenomatosis, salmonellosis (*S. abortusovis*), scrapie; equine
- 10 diseases including contagious equine metritis, dourine, epizootic lymphangitis, equine encephalomyelitis (Eastern and Western), equine infectious anaemia, equine influenza, equine piroplasmosis, equine rhinopneumonitis, equine viral arteritis, glanders, horse mange, horse pox, Japanese encephalitis, surra (*Trypanosoma evansi*), Venezuelan equine encephalomyelitis; swine diseases including atrophic rhinitis of swine,
- 15 enterovirus encephalomyelitis, porcine brucellosis, porcine cysticercosis, porcine reproductive and respiratory syndrome, transmissible gastroenteritis; and lagomorph diseases including myxomatosis, rabbit haemorrhagic disease and tularemia.
- 20 These are some of the List B diseases of OIE; and are transmissible diseases that are considered to be of socio-economic and/or public health importance within countries and that are significant in the international trade of animals and animal products.
- 25 Particular pathogens include foot and mouth disease virus, FeLV (feline leukaemia virus), FIV (feline immunodeficiency virus), CDV (canine distemper virus), Parvovirus, coronavirus, bovine respiratory syncytial virus and bovine viral diarrhoea virus.

In addition, however, antigens are associated with diseases which are not necessarily associated with a pathogen including, for example, cancers where tumour antigens are associated with the disease. Typically, tumour antigens are proteins which are abnormally over-expressed in cancer or
5 which are expressed in an abnormal, eg mutant, form in cancer.

Prions or parts thereof are antigens which may be used in the practice of the invention and are not believed to be associated with pathogenic organisms. Prions are associated with spongiform encephalopathies such as bovine
10 spongiform encephalopathy (BSE) and scrapie in sheep.

Vaccine antigens for use in the context of the present invention include antigenic or immunogenic components of microorganisms such as viruses, bacteria, fungus and parasites including helminths, or parts of such
15 components, intended for the prevention of diseases in animals or that provide protection against diseases in animals.

Suitable antigens include, for example, the E2 protein of bovine viral diarrhoea virus (BVDV), the gp51 protein of bovine leukaemia virus, and
20 the F or G proteins of respiratory syncytial virus (RSV). The amino acid sequences for these proteins are encoded in the viral genomes. The GenBank Accession Nos for the viral genomes are: NC_001781 (human RSV), NC_001989 (bovine RSV), AF033818 (bovine leukaemia virus), AF091605 (type I BVDV) and AF145967 (type II BVDV).

25

Other suitable disease-associated antigens can be selected by the person skilled in the art and typically for many such antigens their amino acid sequence and cDNA sequence encoding them are available in GenBank.

The antigen is typically all or part of a polypeptide associated with a disease such as a polypeptide component of a pathogen or a tumour antigen. The part of the polypeptide may be any part of the polypeptide which is able to elicit an immune response such as an antibody response. It is known that peptides having as few as 5 amino acids may elicit an antibody response, although typically larger peptides are used. Thus, when the antigen is a polypeptide it may have at least 5 amino acids, typically from 5 to 1000 amino acids, such as 5 to 500, 5 to 200, 5 to 100, 5 to 50, 5 to 40, 5 to 30, 5 to 20, for example 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19 or 20 amino acids.

It will be appreciated that the antigen of the compound of the invention may be a variant of a molecule associated with a disease provided that it is able to elicit an immune response which is protective against the disease. Such variants include polypeptides which have one or more amino acid substitutions compared to the native antigen associated with the disease, and as many as 5% substitutions. Typically, the substitutions are conservative substitutions where, for example, a "variant" refers to a protein wherein at one or more positions there have been amino acid insertions, deletions, or substitutions, either conservative or non-conservative, provided that such changes result in a protein whose basic properties, for example enzymatic activity (type of and specific activity), thermostability, activity in a certain pH-range (pH-stability) have not significantly been changed. "Significantly" in this context means that one skilled in the art would say that the properties of the variant may still be different but would not be unobvious over the ones of the original protein.

By "conservative substitutions" is intended combinations such as Gly, Ala; Val, Ile, Leu; Asp, Glu; Asn, Gln; Ser, Thr; Lys, Arg; and Phe, Tyr.

Such variants may be made using standard methods of protein engineering and site-directed mutagenesis.

Preferably the moiety which selectively binds to a dendritic cell and the antigen are covalently linked. When the moiety and antigen are each a polypeptide, the two portions may be linked together by any of the conventional ways of cross-linking polypeptides. For example, the two portions of the compound of the invention are linked together by any of the conventional ways of cross-linking polypeptides, such as those generally described in O'Sullivan *et al Anal. Biochem.* (1979) **100**, 100-108. For example, the first portion may be enriched with thiol groups and the second portion reacted with a bifunctional agent capable of reacting with those thiol groups, for example the N-hydroxysuccinimide ester of iodoacetic acid (NHIA) or N-succinimidyl-3-(2-pyridyldithio)propionate (SPDP), a heterobifunctional cross-linking agent which incorporates a disulphide bridge between the conjugated species. Amide and thioether bonds, for example achieved with m-maleimidobenzoyl-N-hydroxysuccinimide ester, are generally more stable *in vivo* than disulphide bonds.

Further useful cross-linking agents include S-acetylthioglycolic acid N-hydroxysuccinimide ester (SATA) which is a thiolating reagent for primary amines which allows deprotection of the sulphydryl group under mild conditions (Julian *et al* (1983) *Anal. Biochem.* **132**, 68), dimethylsuberimidate dihydrochloride and N,N'-o-phenylenedimaleimide.

Alternatively, the compound may be produced as a fusion compound (or fusion polypeptide) by recombinant DNA techniques whereby a length of DNA comprises respective regions encoding the polypeptide portion of moiety that selectively binds to a dendritic cell and the antigen either

adjacent to one another or separated by a region encoding a linker peptide which does not destroy the desired properties of the compound.

As used herein, a fusion polypeptide is one that contains a polypeptide
5 which is the polypeptide portion of the moiety which binds a dendritic cell fused at the N- or C-terminal end of a polypeptide which is the antigen of the compound of the invention. A simple way to obtain such a fusion polypeptide is by translation of an in-frame fusion of the polynucleotide sequences, *ie* a hybrid gene. The hybrid gene encoding the fusion
10 polypeptide is inserted into an expression vector which is used to transform or transfect a host cell. Transcriptional and translational control regions are typically present in expression vectors. The DNA is then expressed in a suitable host to produce a polypeptide comprising the compound according to the first aspect of the invention.

15 It will be appreciated that the antigen may comprise two or more molecules associated with a disease of the animal or parts or variants of such molecules.

20 In this way, a compound of the invention may be used to vaccinate against more than one disease. Thus, for example, the compound may comprise an antigen from one disease agent (eg the E2 polypeptide of BVDV or an immunogenic portion thereof) and an antigen from a second disease agent (eg the F protein of RSV or an immunogenic agent thereof) in the same
25 polypeptide chain as the moiety which binds a dendritic cell. Other variations will be apparent to the person skilled in the art.

A second aspect of the invention comprises a nucleic acid molecule encoding a fusion compound of the first aspect of the invention.

Suitable nucleic acid molecules may readily be synthesised or constructed by the person skilled in the art using routine methods such as those described in cloning manuals including Sambrook, J. and Russell, D. (2001) "Molecular Cloning: a laboratory manual" 3rd ed. Vol 1-3, Cold Spring Harbor Laboratory Press. Typically the nucleic acid is DNA, but it may be RNA. In the following, where DNA is used, unless the context indicates to the contrary, RNA is also included.

DNA sequences coding for the antigen of the compound of the first aspect of the invention can be identified by searching a database of DNA sequences, such as GenBank and the like. Examples of DNA coding sequences for suitable antigens are given above. Once a DNA sequence coding for the chosen antigen is known, it can be used to design primers and/or probes that are useful in the specific isolation of a DNA or cDNA sequence coding for the antigen, for example from the pathogen associated with the disease to be combated. If a DNA sequence is known, primers and probes can be designed using commercially available software and synthesised by automated synthesis. In general, a DNA sequence coding for the antigen can be isolated from a library of cDNA or DNA sequences generated from an appropriate source, such as the selected pathogen. The library can be screened for the DNA sequences of interest using a probe complementary to a known DNA sequence encoding a selected antigen, preferably under high stringency conditions. DNA sequences that hybridise to the probe can be subcloned and the polypeptide encoded by the DNA sequence can be confirmed by DNA sequence analysis and/or by *in vitro* translation, expression and detection of the polypeptide or like assay. Typically, however, suitable DNA sequences encoding the antigen may be synthesised using the PCR and suitable primers directed at the 5' and 3' ends of the coding region as is well known in the art.

Once the DNA sequence coding for the selected antigen is isolated, it can be operably linked to the DNA sequence encoding the moiety which binds to a dendritic cells, for example HIV gp 120 which selectively binds to DC-SIGN of dendritic cells. The DNA sequence encoding the antigen and the moiety which binds to a dendritic cell are both in the same reading frame operably linked to transcriptional and translational control regions. Transcriptional and translational control regions include promoters, enhancers, cis regulatory elements, polyadenylation sequences, transcriptional and translational initiation regions, and transcriptional termination sequences.

The DNA is then expressed in a suitable host to produce a polypeptide which is a compound of the first aspect of the invention. Thus, the DNA encoding the polypeptide constituting the compound of the invention may be used in accordance with known techniques, appropriately modified in view of the teachings contained herein, to construct an expression vector, which is then used to transform an appropriate host cell for the expression and production of the polypeptide of the invention.

Thus, the DNA encoding the polypeptide constituting the compound of the invention may be joined to a wide variety of other DNA sequences for introduction into an appropriate host. The companion DNA will depend upon the nature of the host, the manner of the introduction of the DNA into the host, and whether episomal maintenance or integration is desired.

Generally, the DNA is inserted into an expression vector, such as a plasmid, in proper orientation and correct reading frame for expression. If necessary, the DNA may be linked to the appropriate transcriptional and translational regulatory control nucleotide sequences recognised by the desired host, although such controls are generally available in the expression vector. The

vector is then introduced into the host through standard techniques. Generally, not all of the hosts will be transformed by the vector. Therefore, it will be necessary to select for transformed host cells. One selection technique involves incorporating into the expression vector a DNA
5 sequence, with any necessary control elements, that codes for a selectable trait in the transformed cell, such as antibiotic resistance. Alternatively, the gene for such selectable trait can be on another vector, which is used to co-transform the desired host cell.

- 10 Host cells that have been transformed by the recombinant DNA of the invention are then cultured for a sufficient time and under appropriate conditions known to those skilled in the art in view of the teachings disclosed herein to permit the expression of the polypeptide, which can then be recovered.

15

Many expression systems are known, including bacteria (for example *E. coli* and *Bacillus subtilis*), yeasts (for example *Saccharomyces cerevisiae*), filamentous fungi (for example *Aspergillus*), plant cells, animal cells and insect cells.

20

- The vectors include a prokaryotic replicon, such as the ColE1 *ori*, for propagation in a prokaryote, even if the vector is used for expression in other, non-prokaryotic, cell types. The vectors can also include an appropriate promoter such as a prokaryotic promoter capable of directing
25 the expression (transcription and translation) of the genes in a bacterial host cell, such as *E. coli*, transformed therewith. Typically, the vector is one which may be stably integrated in a host cell and/or gives high expression of the compound. A suitable vector includes pcDNA 3.1 (+/- His tag) available from Invitrogen.

30

By "promoter" I mean an expression control element formed by a DNA sequence that permits binding of RNA polymerase and transcription to occur. Promoter sequences compatible with exemplary hosts are typically provided in plasmid vectors containing convenient restriction sites for
5 insertion of a DNA segment of the present invention.

Another aspect of the invention therefore provides a host cell transformed with a nucleic acid encoding the compound according to the first aspect of the invention.

10 Particularly preferred host cells for expression of the compound of the invention include COS and CHO cells which are able to glycosylate proteins. *E. coli* cells may be used and in that case, the lipopolysaccharide component which may be present in the final product of the compound may
15 in itself be an immunostimulant. This may be beneficial in some circumstances, but may be undesirable in others where it may compromise the measurement of an immune response.

Culturing of the host cell under conditions permitting expression of the
20 DNA polymer is carried out conventionally. The product may be recovered by conventional methods according to the host cell and according to the localisation of the expression product (intracellular or secreted into the culture medium or into the cell periplasm). Thus, where the host cell is bacterial, such as *E. coli* it may, for example, be lysed physically,
25 chemically or enzymatically and the protein product isolated from the resulting lysate. Where the host cell is mammalian, the product may generally be isolated from the nutrient medium or from cell free extracts. Where the host cell is a yeast such as *Saccharomyces cerevisiae* or *Pichia pastoris*, the product may generally be isolated from lysed cells or from the
30 culture medium, and then further purified using conventional techniques.

The specificity of the expression system may be assessed by western blot using an antibody directed against the polypeptide of interest.

Conventional protein isolation techniques include selective precipitation
5 adsorption chromatography, and affinity chromatography including a monoclonal antibody affinity column.

It will be appreciated that the invention readily lends itself to the provision of a "cassette expression" vector whereby a vector is produced which
10 contains a coding sequence for the moiety which binds a dendritic cell, such as HIV-1 gp 120, which can readily be fused to a coding region for the chosen antigen which is inserted at an appropriate place in the cassette expression vector.

15 Thus, a still further aspect of the invention provides a nucleic acid molecule comprising (i) a portion which encodes the moiety which selectively binds to a dendritic cell and (ii) an insertion point for insertion of a polynucleotide encoding an antigen wherein when said polynucleotide is inserted into said insertion point, said nucleic acid molecule encodes a compound according
20 to the first aspect of the invention.

Typically, the nucleic acid molecule takes the form of an expression vector which contains appropriate transcriptional and translational control signals to allow expression of the fusion polypeptide once the antigen coding
25 region has been inserted into the insertion point. As is well known in the art, the insertion point in a "cassette expression" vector is one which readily allows for the insertion of the appropriate coding sequence (one encoding an antigen in this case) so that an inframe fusion is produced. Typically, the insertion point is a unique restriction site within the nucleic acid.

Typically, the vector is one which may be stably integrated in a host cell and/or gives a high level of expression of the compound. It may, conveniently be based on the pcDNA3.1 (+/- His tag) vector available from Invitrogen.

5

Since the antigen may be fused to the binding moiety at its N-terminus or C-terminus, the insertion point may be at the 5' end or 3' end of the coding sequence for the binding moiety, and suitable transcription and translation signals placed appropriately as will be known to the person skilled in the art.

10

The compound of the first aspect of the invention is useful in the immunisation against, and treatment of, diseases in animals. Thus, the compound is useful in a vaccine.

15

A further aspect of the invention provides a compound according to the first aspect of the invention for use in medicine. Typically, the compound is packaged and presented as a medicament for use in an animal.

20

A still further aspect of the invention provides a vaccine comprising a compound according to the first aspect of the invention is preferably adjuvanted in the vaccine formulation of the invention. Suitable adjuvants are commercially available such as alum and Quil A.

25

It is preferably that the adjuvant composition induces an immune response predominantly of the Th1 type. High levels of Th1-type cytokines (for example, IFN- γ , TNF α , IL-2 and IL-12) tend to favour the induction of cell mediated immune responses to an administered antigen. Preferably, the compound may give rise to both a Th1 and Th2 responses.

A still further aspect of the invention provides a pharmaceutical composition comprising a compound according to the first aspect of the invention and a pharmaceutically acceptable carrier.

5

The carrier(s) must be "acceptable" in the sense of being compatible with the compound of the invention and not deleterious to the recipients thereof. Typically, the carriers will be water or saline which will be sterile and pyrogen free. However, other acceptable carriers may be used.

10

Typically, the pharmaceutical compositions or formulations of the invention are for parenteral administration, more particularly for intravenous administration. The compositions or formulations may be administered by the following routes: intramuscular, subcutaneous, intradermal, intranasal, intravenous, oral, and intraperitoneal. Intramuscular is most preferred as it is the most practical way for a vet.

15

Formulations suitable for parenteral administration include aqueous and non-aqueous sterile injection solutions which may contain anti-oxidants, buffers, bacteriostats and solutes which render the formulation isotonic with the blood of the intended recipient; and aqueous and non-aqueous sterile suspensions which may include suspending agents and thickening agents.

20

The invention also provides a method of immunising an animal against a disease comprising the step of administering to the animal a compound according to the first aspect of the invention.

25

It will be appreciated that the compound administered is selected on the basis of (i) the animal to be immunised (so that an appropriate binding

moiety can be chosen, which is foreign to the animal) and (ii) the disease to be immunised against (so that an appropriate antigen is chosen).

Thus, a particularly preferred embodiment of the invention is a method of
5 immunising an animal against a disease comprising administering to the animal a compound comprising (i) a moiety which selectively binds to a dendritic cell in said animal but which does not naturally occur in said animal and (ii) an antigen relevant to said disease.

10 The methods and compositions may be directed towards immunising and protecting animals, preferably animals of economic importance, including farm animals such as cows, sheep, goats, pigs, horses and rabbits, and companion animals, such as cats and dogs. Depending on the type of antigens used, immunisation of animals with these antigens can result in the
15 prevention of infection, amelioration of symptoms, decrease in mortality and/or induction of neutralising antibodies.

Thus, the invention also provides a method of combating a disease in an animal comprising administering to the animal a compound according to the
20 first aspect of the invention.

A particularly preferred embodiment is a method of combating a disease in an animal comprising the step of administering to the animal a compound comprising (i) a moiety which selectively binds to a dendritic cell in said
25 animal but which does not naturally occur in said animal and (ii) an antigen relevant to said disease.

Typically, the compound is administered as an immunogenic composition.

Diseases which may be immunised against or combated according to the methods of the invention include BVDV, RSV and bovine leukaemia virus and appropriate antigens may be selected for example from the BVDV E2 protein, the RSV F and G proteins and gp51 of bovine leukaemia virus.

5 Thus, the compound of the invention may be used prophylactially or therapeutically.

Typically, the disease to be treated is one caused by a pathogen. However, tumours may also be treated.

10

The tumours that may be treated by the methods of the invention include melanomas (see, for example, Nestle, F.O. (2002) *Clinical & Experimental Dermatology* 27(7), 597-601). Typically, more than one antigen may be needed in order to be effective. These may be combined in the same compound for vaccination or in separate such compounds. Typically, the

15

antigens may be mutated antigens (eg mutated p16 (CDKN2A)), shared tumour specific antigens (eg MAGE-1, MAGE-3 and NY-ESO-1), differentiation antigens (eg tyrosinase, gp100 and MelanA/MART-1) or cell surface gangliosides (eg GM2, GD2 and GD3) as described in Nestle (2002)

20

supra.

The term "tumour" is to be understood as referring to all forms of neoplastic cell growth, including tumours of the lung, liver, blood cells, skin, pancreas, stomach, colon, prostate, uterus, breast, lymph glands and bladder. Solid

25

tumours are especially suitable.

Still further aspects of the invention provide the use of a compound according to the first aspect of the invention in the manufacture of a medicament for combating a disease in an animal; or in the manufacture of

30

a vaccine for immunising an animal.

Particularly preferred embodiments include the use of a compound comprising (i) a moiety which selectively binds to a dendritic cell in an animal but which does not naturally occur in said animal and (ii) an antigen relevant to a disease in said animal in the manufacture of a medicament for combating the disease in the animal; and the use of a compound comprising (i) a moiety which selectively binds to a dendritic cell in an animal but which does not naturally occur in said animal and (ii) an antigen relevant to a disease in said animal in the manufacture of a vaccine for immunising the animal.

The invention particularly includes the use of a compound for vaccination of an animal comprising (i) a moiety selected from any of HIV gp120, the LAM protein of *Mycobacterium tuberculosis* or a glycoprotein of Ebola virus, or parts thereof, and (ii) an antigen in the manufacture of a medicament for combating disease associated with the antigen or in the manufacture of a vaccine for immunising the animal against the disease associated with the antigen.

Animals vaccinated using the method of the invention can be distinguished from animals naturally infected with the disease agent with which the antigen of the compound of the invention is associated since vaccinated animals will have had an immune response to the moiety which binds to a dendritic cell whereas naturally-infected animals will not.

Thus, a further aspect of the invention provides a method determining whether an animal has been administered a compound according to the first aspect of the invention, the method comprising determining whether the animal has had an immune response to the moiety which selectively binds

to a dendritic cell. It may also be useful to determine whether the animal has had an immune response to the antigen present in the compound.

Typically, an antibody-containing sample is taken from the animal and it is determined whether an antibody directed at the said moiety is present. Whether an antibody directed at said antigen is present may also be determined. Typically, the sample is a blood sample. Typically, the blood sample is obtained from the jugular vein or tail vein of the animal.

- 10 The invention also provides kits of parts which may be used to distinguish naturally-infected animals and those administered a compound of the first aspect of the invention.

- 15 One kit of parts comprises (i) a compound according to the first aspect of the invention and (ii) means for detecting an immune response to the moiety present in the compound which selectively binds to a dendritic cell, and/or (iii) means for detecting an immune response to the antigen in said compound.

- 20 A further kit of parts comprises (i) means for detecting an immune response to an animal disease antigen and (ii) means for detecting an immune response for a moiety which selectively binds to a dendritic cell.

- 25 In a preferred embodiment, the binding moiety is HIV-1 gp 120, or a part thereof.

Typically, an immune response is detected by determining whether or not a sample from the animal contains appropriate antibodies (ie to the antigen and/or the binding moiety).

Thus, suitable means for detecting the immune response or responses include using an ELISA to determine an antibody response. An ELISA for measuring gp120 antibody response are commercially available, for example from Advanced BioSciences Laboratories Ltd or
5 ImmunoDiagnostics Inc.

The invention will now be described in more detail by reference to the following Figures and Examples.

10 Figure 1 shows the partial cDNA sequence encoding bovine DC-SIGN. The letter "n" means any of the four naturally occurring nucleotides A, G, C or T.

Figure 2 shows the alignment of cDNA sequences encoding DC-SIGN from
15 chimp, Pan, human, Macaca and mouse using the SE Central sequence analysis package (Align Plus and Clone Manager) from Scientific and Educational Software.

The alignment, parameters and settings are indicated. Dashes indicate no
20 nucleotides.

Figure 3 shows the alignment of deduced amino acid sequence for bovine DC-SIGN (partial sequence) against amino acid sequence for DC-SIGNs from chimp, human and macaca. Dashes indicate no amino acids. The
25 asterisks in the bovine DC-SIGN amino acid sequence are unallocated amino acids. The alignments were done using the SE Central sequence analysis package. The Alignment parameters were: Global Protein alignment against reference molecule; Parameters: Scoring matrix: BLOSUM 62; Reference molecule putative boDC-SIGN, region 1-577;
30 Number of sequences to align 4. Putative boDC-SIGN 192 amino acids;

chimp_DC-SIGN 404 amino acids; huDC_SIGN_CDS 405 amino acids;
Macaca mulatta_CDS 405 amino acids.

Example 1: Ability of gp120 protein to bind to DC-SIGN on bovine cells

To assess the ability of HIV-1 gp120 protein to bind to DC-SIGN on bovine cells, cDNA encoding the protein is cloned into an expression vector containing a His-tag (ie oligo histidine tag). As a control, an unrelated His-tagged protein that is known not to bind to DC-SIGN, such as FITC-labelled ovalbumin (OVA), is used. To follow the uptake of gp120-His by DC, a DC culture is prepared and divided into 4 groups of cells. First (experimental) and second (control) groups are pulsed with either gp120-His or an unrelated His-tagged protein for 2 hours and washed. The third (negative control) group is only treated with growth medium, whereas the fourth group (uptake control) is used to demonstrate the ability of DC to take up FITC-labelled antigen (in this case OVA) as a functional control for the ability of DC to take up antigen. Subsequently, all groups of cells are used to observe the cellular localisation pattern of proteins by confocal fluorescent microscopy, using a FITC-labelled anti-His antibody. Finally, polyclonal antibodies are raised against the bovine DC-SIGN molecule to show the specificity of gp120-His uptake via DC-SIGN by pre-incubating DC with these antibodies to block DC-SIGN. Blocking of DC-SIGN should reduce uptake of gp120-His, but not any other molecules or the control.

Results

Based on the current similarities on published DC-SIGN sequences, it is expected that the gp120-His will bind and subsequently be internalised via the bovine DC-SIGN molecule, and this binding/uptake will be blocked by

polyclonal antibodies, whereas this will have no effect on the controls used.

Discussion

5 To discriminate between bound and internalised gp120-His, one can measure the amount of gp120-His taken up by the cell, cells will be treated with EDTA-trypsin solution to degrade gp120-His bound on the surface, but not internalised. Thereafter, cellular and cytosolic extracts can be analysed for the presence of gp120-His by western blotting, either using a
10 monoclonal antibody to the His-tag or directly to the gp120 protein. Upon successful uptake of gp120-His, gp120 is expressed together with model-antigens (*ie* envelope proteins of bovine respiratory syncytial virus or bovine viral diarrhoea virus E2 protein) which will be used for further studies (for the sake of simplicity, this product is termed gp120-Ag in
15 further examples).

Example 2: Enhanced development of a Th1-type immune response by antigen-pulsed DC

20 Recent data emphasise that uptake of antigens via pattern recognition receptors, such as DC-SIGN, strongly enhances the development of a Th1-type of immune response with IFN α and IL-12 being released by antigen-pulsed DC. The release of these cytokines favours the development of a cell-mediated immunity. To demonstrate the effect of gp120-His or gp120-Ag on bovine DC, cells are incubated for 2h with different doses of gp120-
25 His or gp120-Ag. After this time, the supernatants are harvested and analysed for the presence of IFN α and IL-12 using ELISA-systems.

Results

It is expected that the amount of IFN α and IL-12 released by DC exposed to gp120-His or gp120-Ag should be greater than that produced by peripheral blood lymphocytes or macrophages exposed for the same time and amount to gp120-His.

Example 3: Stimulation of naïve T-cells by DC

In contrast to other antigen-presenting cells (APC), such as macrophages and B cells, DC have the unique capacity to stimulate naïve T cells. To evaluate the stimulatory capacity of gp120-pulsed DC, different subsets of APC are generated and incubated with gp120-Ag. After this time, DC are inactivated by exposure to mitomycin-D and added in various relations to sorted CD4⁺ T cells of the same donor. After 5 days in culture, the stimulatory capacity of gp120-Ag pulsed DC, macrophages and B cells are evaluated by measuring the amount of [³H]-labelled thymidine incorporated in the DNA of proliferating T cells by liquid scintillation counting using a beta-counter.

It is expected that strong T cell proliferation is observed only in the presence of DC, not macrophages or B cells (which show a 10-fold lower response).

Example 4: Endocytosis of DC-SIGN as a result of HIV-1 binding

In the human system, endocytosis of DC-SIGN as a result of HIV-1 binding decreases the number of DC-SIGN molecules on the DC surface. Specific monoclonal anti-DC-SIGN antibodies are raised in the mouse by standard methods and are tested for whether they interfere with gp120-Ag binding to

DC-SIGN. A non-interfering antibody and a fluorescent-tagged secondary anti-mouse antibody are used to quantify DC-SIGN expression on the surface of DC (pulsed with gp120-Ag medium as negative control) with the help of Fluorescence Activated Cell Sorting (FACS). The measurement is
5 repeated on a similar group of DC after pulsing with gp120-Ag for 2 hours (experimental group). Sufficient amount of gp120-Ag should be used to increase the likelihood of DC-SIGN binding and internalisation.

Results

10

If the amount of surface DC-SIGN in the experimental group is similar to that of the control group, it is expected that probably no significant endocytosis of DC-SIGN occurs. If surface DC-SIGN expression is reduced upon gp120-Ag binding, DC-SIGN is most likely endocytosed by
15 DC.

It is necessary for endocytosis to occur for an immune response to occur.

Example 5: Effect of gp120-Ag immunisation in animals

20

To demonstrate the effect of a gp120-Ag immunisation in animals, cattle are immunised with different doses of gp120-Ag, and the development of an antibody response to either the Ag or gp120 is analysed using ELISA-systems available for the specific Ag or commercially available for gp120.
25 Four groups of animals are immunised either with the carrier solution alone, purified gp120-His, purified Ag, or gp120-Ag. Blood samples are taken on a weekly base before and after immunisation, and the antibody titre measured in serum samples.

Results

It is expected that animals immunised with the gp120-Ag will develop antibodies to both, the gp120 as well as the Ag of interest. To estimate the relation between Ag-specific antibodies and gp120-specific antibodies, the antibody-titre of animals immunised with the gp120-His will serve as control, thus establishing data to demonstrate the efficiency of gp120-Ag being taken up and presented.

Typically, the antibody titre to gp120 in animals immunised with the gp120-Ag can be used as a direct indicator for the successful immunisation of an individual animal, and will also allow the discrimination of immunised versus naturally infected animals.

Example 6: Challenging successfully immunised animals with antigen

Having established the successful immunisation of animals, the same groups of animals are challenged with the antigen, and the development of clinical signs monitored.

Results

It is expected that immunised animals should have a protection against the subsequent challenge and should not develop any clinical symptoms, whereas animals given the gp120-His or carrier solution should do so.

Example 7: Cloning of bovine DC-SIGN cDNA

Bovine dendritic cells were isolated according to the procedure in Werling *et al* (2002) *J. Leukoc. Biol.* **72**, 297-304, and mRNA isolated. A

SMART™ RACE cDNA amplification kit from Becton Dickinson (Cat. No. K1811-1) was used to amplify cDNA using the primer TAGCTGACTCCTTGTCCAAGTG. The amplified cDNA was sequenced and the nucleotide sequence is given in Figure 1.

CLAIMS

1. A compound for vaccination of an animal comprising (i) a moiety which selectively binds to a dendritic cell in the animal but which moiety
5 does not naturally occur in the animal and (ii) an antigen.
2. A compound according to Claim 1 wherein the moiety selectively binds to a pattern recognition receptor on a dendritic cell.
- 10 3. A compound according to Claim 2 wherein the pattern recognition receptor is DC-SIGN.
4. A compound according to Claims 1 or 2 wherein the moiety which selectively binds is a protein.
- 15 5. A compound according to Claim 4 wherein the moiety which selectively binds is any one of HIV gp 120, the LAM protein of *Mycobacterium tuberculosis* or a glycoprotein of Ebola virus, or parts thereof.
- 20 6. A compound for vaccination of an animal comprising (i) a moiety selected from any of HIV gp120, the LAM protein of *Mycobacterium tuberculosis* or a glycoprotein of Ebola virus, or parts thereof, and (ii) an antigen.
- 25 7. A compound according to any one of the preceding claims wherein the antigen is a polypeptide.

8. A compound according to any one of the preceding claims wherein the antigen is a molecule associated with a disease of the animal or part or variant of such a molecule.

5 9. A compound according to any one of the preceding claims wherein the antigen comprises two or more molecules associated with a disease of the animal or parts or variants of such molecules.

10 10. A compound according to Claims 8 or 9 wherein the antigen is an antigenic component of a pathogen or a tumour or an antigenic part or variant of such a component.

11. A compound according to Claim 10 wherein the pathogen is any of a bacterium, virus, fungus, protozoa or helminth.

15

12. A compound according to Claim 11 wherein the antigen is an antigenic component of a pathogen selected from pathogens associated with OIE list A diseases, or a part or variant of such a component.

20 13. A compound according to any one of the preceding claims wherein the moiety and the antigen are covalently linked.

14. A compound according to any one of the preceding claims wherein the moiety which selectively binds, and the antigen, each comprise a polypeptide and both are present in the same polypeptide chain.

25

15. A nucleic acid molecule encoding a compound according to Claim 14.

16. An expression vector comprising a nucleic acid molecule according to Claim 15.

17. A host cell comprising a nucleic acid molecule according to Claim 15 or an expression vector according to Claim 16.

18. A vaccine comprising a compound according to any one of Claims 1 to 14 or a nucleic acid molecule according to Claim 15.

19. A vaccine according to Claim 18 further comprising an adjuvant.

20. A compound according to any one of Claims 1 to 14 or a nucleic acid molecule according to Claim 15 for use in medicine.

21. A pharmaceutical composition comprising a compound according to any one of Claims 1 to 14 or a nucleic acid molecule according to Claim 15 and a pharmaceutically acceptable carrier.

22. A method of immunising an animal against a disease comprising the step of administering to the animal a compound according to any one of Claims 1 to 14 or a nucleic acid molecule according to Claim 15.

23. A method of combating a disease in an animal comprising the step of administering to the animal a compound according to any one of Claims 1 to 14 or a nucleic acid molecule according to Claim 15.

24. The method according to any one of Claims 22 or 23 wherein the disease is one caused by a pathogen.

25. The method according to Claim 24 wherein the pathogen is any of a bacterium, virus, fungus, protozoa or helminth.

26. The method according to Claim 25 wherein the pathogen is one
5 associated with OIE List A diseases.

27. The method according to any one of Claim 22 or 23 wherein the animal is a mammal.

10 28. The method according to any one of Claims 22 to 27 wherein the animal is a companion animal or farm animal.

29. The method according to any one of Claims 27 or 28 wherein the animal is a cow, sheep, horse, pig, goat, dog, cat or rabbit.

15

30. Use of a compound according to any one of Claims 1 to 14 or a nucleic acid according to Claim 15 in the manufacture of a medicament for combating a disease in an animal.

20 31. Use of a compound according to any one of Claims 1 to 14 or a nucleic acid according to Claim 15 in the manufacture of a vaccine for immunising an animal.

32. Use according to Claims 30 or 31 wherein the disease is one caused
25 by a pathogen.

33. Use according to Claim 32 wherein the pathogen is any of a bacterium, virus, fungus, protozoa or helminth.

34. Use according to Claim 33 wherein the pathogen is one associated with OIE List A diseases.

35. Use according to Claims 30 or 31 wherein the animal is a mammal.

36. Use according to Claims 30 or 31 wherein the animal is a companion animal or farm animal.

37. Use according to Claims 35 or 36 wherein the animal is a cow, sheep, horse, pig, goat, dog, cat or rabbit.

38. A method of making a compound according to Claim 1 or 6 comprising linking the said moiety and the said antigen.

39. A method of making a compound according to Claim 14 which comprises a polypeptide, said method comprising (i) culturing host cell according to Claim 17 which expresses said polypeptide and (ii) isolating said polypeptide.

40. A method of making a nucleic acid according to Claim 15 comprising linking a nucleic acid molecule which encodes a moiety which selectively binds to a dendritic cell and a nucleic acid molecule which encodes an antigen.

41. A nucleic acid molecule comprising (i) a portion which encodes a moiety which selectively binds to a dendritic cell and (ii) an insertion point for insertion of a polynucleotide encoding an antigen wherein when said polynucleotide is inserted into said insertion point, said nucleic acid molecule encodes a compound according to Claim 14.

42. The nucleic acid according to Claim 41 wherein said nucleic acid encodes a moiety which selectively binds to a pattern recognition receptor on a dendritic cell.

5 43. The nucleic acid according to Claim 42 wherein the recognition receptor is DC-SIGN.

44. The nucleic acid according to Claims 41 or 42 wherein the moiety which selectively binds is any one of HIV gp 120, the LAM protein of
10 *Mycobacterium tuberculosis* or a glycoprotein of Ebola virus.

45. A method of determining whether an animal has been administered a compound according to Claim 1, the method comprising determining whether the animal has had an immune response to said moiety which
15 selectively binds to a dendritic cell.

46. A method according to Claim 45 comprising the further step of determining whether the animal has had an immune response to the antigen present in said compound.

20

47. A kit of parts comprising (i) a compound according to any one of Claims 1 to 14 or a nucleic acid molecule according to Claim 15 and (ii) means for detecting an immune response to the moiety present in said compound which selectively binds to a dendritic cell, and/or (iii) means for
25 detecting an immune response to the antigen present in said compound.

48. A kit of parts according to Claim 47 wherein if present part (ii) comprises all, or a portion of, said moiety which binds to an antibody raised against said moiety and part (iii) comprises all, or a portion, of said antigen
30 which binds to an antibody raised against said antigen.

49. A kit of parts comprising (i) means for detecting an immune response to an animal disease antigen and (ii) means for detecting an immune response to a moiety which selectively binds to a dendritic cell.

5

50. A kit of parts according to any one of Claims 47 to 49 wherein the means for detecting an immune response is an ELISA.

51. Any novel method of vaccinating an animal wherein the so-
10 vaccinated animal can be distinguished from a naturally infected animal as described herein.

ABSTRACT**ANTIGEN DELIVERY SYSTEM**

- 5 A compound for vaccination of an animal comprising (i) a moiety which selectively binds to a dendritic cell in the animal but which does not naturally occur in the animal and (ii) an antigen.

Typically, the moiety which selectively binds as said binds to DC-SIGN.

- 10 The moiety may be HIV-1 gp120.

The compound of the invention may be used to vaccinate animals and, following vaccination, they may be distinguished from naturally infected animals.

FIGURE 1.

FIGURE 1

ctccttgtcc aagtgtccaa ggtccccagc tccntaagtc aggaacaatc cangcaagac	60
gcgatctacc agaacctgac ccagcttaaa gctgcagtgg gtgagctctc agagaaatcc	120
aagctgcagg agatctacca ggagctgacc cagctgaagg ctgcagtggg tgagcttcca	180
gagaaatcna agcagcagga gatctaccag gagctgaccc ggctgaaggc tgcagtgggt	240
gagcttccag agaaatcnaa gctgcaggag atctaccagg agctgaccng gctgaaggct	300
gcagtgggtg agcttccaga gaaatctaag atgcaggaga tctaccagga gctgacncgg	360
ctgaaggctg cagtgggtga gctcccagag aaatctaagc agcaggagat ctaccaggag	420
ctgacccggc tgaaggctgc agtgggtgag ctaccagaga aatctaagca gcaggagatc	480
taccaggagc tgacccggct gaaggctgca gtgggtgagc ttccagataa atccaagcag	540
caggagatct accaggagct gaccagctg aaggctg	577

FIGURE 2 (Page 1 of 4)

Alignment Results

Alignment: Multi-way DNA alignment.

Parameters: Scoring matrix: Linear (Mismatch 2, OpenGap 4, ExtGap 1)

Number of sequences to align: 5

Pos	Sequence	Start	End	Length	Matches	%Matches
1	Chimp_DC-SIGN	1	1212	1212	1203	99
2	Pan troglodytes	1	1212	1212	1203	99
3	huDC-SIGN_CDS	1	1215	1215	1203	99
4	Macaca mulatta_CDS	1	1215	1215	1133	93
5	mu_DC-SIGN.txt	1	1450	1450	670	44

FIGURE 2 (Page 2 of 4)

Alignment Results

Alignment: Multi-way DNA alignment.

Parameters: Scoring matrix: Linear (Mismatch 2, OpenGap 4, ExtGap 1)

Number of sequences to align: 5

Settings: Similarity significance value cutoff: >= 60%

Summary of Percent Matches:

Sequence	1:	Chimp DC-SIGN	1 -	1212	(1212 bps)	99%
Sequence 2:	Pan troglodytes	1 -	1212	(1212 bps)	99%	
Sequence 3:	huDC-SIGN CDS	1 -	1215	(1215 bps)	99%	
Sequence 4:	Macaca mulatta CDS	1 -	1215	(1215 bps)	93%	
Sequence 5:	mu_DC-SIGN.txt	1 -	1450	(1450 bps)	44%	

```

Chimp DC-SIG      1 -----
Pan troglody      1 -----
huDC-SIGN CD      1 -----
Macaca mulat      1 -----
mu_DC-SIGN.t      1 gggcactgagaagtggctgtgaaacaggggtgttctaaaggaatggggaagagggaact

Chimp DC-SIG      36 gggcctcctggaggagggaacagctgaga-----ggcct
Pan troglody      36 gggcctcctggaggagggaacagctgaga-----ggcct
huDC-SIGN CD      36 gggcctcctggaggagggaacagctgaga-----ggcct
Macaca mulat      36 ggaacctcctggaggagggaacagctggga-----ggcgt
mu_DC-SIGN.t      61 tcgccctctggtgagggaactgctgacatccagccacaccaggcactccatcaaaggctt

Chimp DC-SIG      69 tggattccgacagactcgagggtacagagcttagcagggtgtcttggccaaggtc---
Pan troglody      69 tggattccgacagactcgagggtacagagcttagcagggtgtcttggccaaggtc---
huDC-SIGN CD      69 tggattccgacagactcgagggtacagagcttagcagggtgtcttggccaaggtc---
Macaca mulat      69 tggattccgacagactcgagggtacagagcttagcagggtgtcttggccaaggtc---
mu_DC-SIGN.t      121 tggcttccaaacaaattctggattcagtagctcacagggtgtcctggtccacagteaagt

Chimp DC-SIG      125 --cctggtgctgcaactcctctccttccac-----gctcttgggtgggctcct
Pan troglody      125 --cctggtgctgcaactcctctccttccac-----gctcttgggtgggctcct
huDC-SIGN CD      125 --cctggtgctgcaactcctctccttccac-----gctcttgggtgggctcct
Macaca mulat      125 --cctggtgctgcaactcctctccttccac-----gctcttgggtgggctcct
mu_DC-SIGN.t      181 ccepttggcacttgcagggtgcttgcagggtgttgttctgtggtgcttgccttgcctt

Chimp DC-SIG      171 tggcctcctggaagagggaacagctgaga-----ggcct
Pan troglody      171 tggcctcctggaagagggaacagctgaga-----ggcct
huDC-SIGN CD      171 tggcctcctggaagagggaacagctgaga-----ggcct
Macaca mulat      171 tggcctcctggaagagggaacagctgaga-----ggcct
mu_DC-SIGN.t      241 tggcctcctggaagagggaacagctgaga-----ggcct

Chimp DC-SIG      231 ctacaggaactgagtcagagctgaggtgagctctcagagaaatccagct
Pan troglody      231 ctacaggaactgagtcagagctgaggtgagctctcagagaaatccagct
huDC-SIGN CD      231 ctacaggaactgagtcagagctgaggtgagctctcagagaaatccagct
Macaca mulat      231 ctacaggaactgagtcagagctgaggtgagctctcagagaaatccagct
mu_DC-SIGN.t      295 ctacaggaactgagtcagagctgaggtgagctctcagagaaatccagct

Chimp DC-SIG      291 ctacaggaactgagtcagagctgaggtgagctctcagagaaatccagct
Pan troglody      291 ctacaggaactgagtcagagctgaggtgagctctcagagaaatccagct
huDC-SIGN CD      291 ctacaggaactgagtcagagctgaggtgagctctcagagaaatccagct
Macaca mulat      291 ctacaggaactgagtcagagctgaggtgagctctcagagaaatccagct
mu_DC-SIGN.t      339 ctacaggaactgagtcagagctgaggtgagctctcagagaaatccagct

Chimp DC-SIG      349 ctacaggaactgagtcagagctgaggtgagctctcagagaaatccagct
Pan troglody      349 ctacaggaactgagtcagagctgaggtgagctctcagagaaatccagct
huDC-SIGN CD      349 ctacaggaactgagtcagagctgaggtgagctctcagagaaatccagct
Macaca mulat      349 ctacaggaactgagtcagagctgaggtgagctctcagagaaatccagct
mu_DC-SIGN.t      392 ctacaggaactgagtcagagctgaggtgagctctcagagaaatccagct

Chimp DC-SIG      397 ctacaggaactgagtcagagctgaggtgagctctcagagaaatccagct
Pan troglody      397 ctacaggaactgagtcagagctgaggtgagctctcagagaaatccagct
huDC-SIGN CD      397 ctacaggaactgagtcagagctgaggtgagctctcagagaaatccagct
Macaca mulat      397 ctacaggaactgagtcagagctgaggtgagctctcagagaaatccagct
mu_DC-SIGN.t      449 ctacaggaactgagtcagagctgaggtgagctctcagagaaatccagct

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FIGURE 3 (PAGE 1 OF 2)

putative boDC-SIGN	-----LLV--
Chimp_DC-SIGN	MSDSKEPRLQQLGLLEEEQLRGLGFRQNRGYKSLAGCLGHGPLVLQLLSFTLLAGLLV--
huDC-SIGN_CDS	MSDSKEPRLQQLGLLEEEQLRGLGFRQNRGYKSLAGCLGHGPLVLQLLSFTLLAGLLV--
Macaca mulatta_CDS	MSDSKEPRLQQLDLLEEEQLGGVGFRQNRGYKSLAGCLGHGPLVLQLLSFTLLAGLLV--
putative boDC-SIGN	-----
Chimp_DC-SIGN	-----QVSKVPSS*SQE--QS*QDAIYQNL-----
huDC-SIGN_CDS	-----QVSKVPSSISQE--ESRQDVYQNL-----
Macaca mulatta_CDS	-----QVSKVPSSISQE--QSRQDAIYQNL-----
	-----QVSKVPSSISQG--QSKQDAIYQNL-----
putative boDC-SIGN	-----
Chimp_DC-SIGN	-----TQLKAAVG-----ELSEKSKLQEIYQE-----LTQLKAAVGEPEK*--
huDC-SIGN_CDS	-----TQLKAAVG-----ELSEKSKLQEIYQE-----LTQLKAAVGEPEKS--
Macaca mulatta_CDS	-----TQLKAAVG-----ELSEKSKLQEIYQE-----LTQLKAAVGEPEKS--
	-----TQLKVAVS-----ELSEKSKQEIYQE-----LTRLKAAVDELPEKS--
putative boDC-SIGN	-----
Chimp_DC-SIGN	-----KQEIYQEL-----KQEIYQEL
huDC-SIGN_CDS	-----KQEIYQEL-----KQEIYQEL
Macaca mulatta_CDS	-----KLQEIYQEL-----KLQEIYQEL
	-----KQEIYEEL-----KQEIYEEL
putative boDC-SIGN	-----
Chimp_DC-SIGN	-----EIIY-QEILT*LKAAVGEPEL-----EIIY-QEILT*LKAAVGEPEL-----
huDC-SIGN_CDS	-----EIIY-QEILT*TRKAAVGEPEL-----EIIY-QEILT*TRKAAVGEPEL-----
Macaca mulatta_CDS	-----EIIY-QEILT*TRKAAVGEPEL-----EIIY-QEILT*TRKAAVGEPEL-----
	-----EIIY-QEILT*TRKAAVGEPEL-----EIIY-QEILT*TRKAAVGEPEL-----
putative boDC-SIGN	-----
Chimp_DC-SIGN	-----EKSKMQEIYQEL*R-LKAAVGE*P-----EKSKMQEIYQELTR-LKAAVGEPEL
huDC-SIGN_CDS	-----EKSKMQEIYQELTR-LKAAVGEPEL-----EKSKMQEIYQELTR-LKAAVGEPEL
Macaca mulatta_CDS	-----EKSKMQEIYQELSR-LKAAVGEPEL-----EKSKMQEIYQELSR-LKAAVGEPEL

FIGURE 3 (PAGE 2 OF 2)

putative boDC-SIGN	EKSKQQE-----IYQELTR-----
Chimp_DC-SIGN	EKSKQQE-----IYQELTR-----
huDC-SIGN_CDS	EKSKQQE-----IYQELTR-----
Macaca mulatta_CDS	EKSKQQE-----IYQELTR-----
putative boDC-SIGN	LKAAVGE*PEKS-----KQEIYQELT*LKAAVGELP***--KQQE-----
Chimp_DC-SIGN	LKAAVGELPEKS-----KQEIYQELTRLKAAVGELP--EKSQQE-----
huDC-SIGN_CDS	LKAAVGELPEKS-----KQEIYQELTRLKAAVGELP--EKSQQE-----
Macaca mulatta_CDS	LKAAVGDLPEKS-----KQEIYQKLTLKAAVDGLP--DRSKQQE-----
putative boDC-SIGN	-----IYQELTQLKA-----
Chimp_DC-SIGN	-----IYQELTQLKAAVERLCRRCPW-----
huDC-SIGN_CDS	-----IYQELTQLKAAVERLCHPCPW-----
Macaca mulatta_CDS	-----IYQELIQLKAAVERLCHPCPW-----
putative boDC-SIGN	-----
Chimp_DC-SIGN	EWTFQGNFCYFMSNSQRNWHDSITACKEVGAQLVVIKSABEONFLQLQSSRNRFWTMGL
huDC-SIGN_CDS	EWTFQGNFCYFMSNSQRNWHDSITACKEVGAQLVVIKSABEONFLQLQSSRNRFWTMGL
Macaca mulatta_CDS	EWTFQGNFCYFMSNSQRNWHDSITACQEVGAQLVVIKSABEONFLQLQSSRNRFWTMGL
putative boDC-SIGN	-----
Chimp_DC-SIGN	SDLNHEGTWQWVDGSPLLPSFKQYWNRGEPNNVGEEDCAEFSGNGWDDKCNLAKFWICK
huDC-SIGN_CDS	SDLNHEGTWQWVDGSPLLPSFKQYWNRGEPNNVGEEDCAEFSGNGWDDKCNLAKFWICK
Macaca mulatta_CDS	SDLNHEGTWQWVDGSPLLPSFKQYWNKGEPNNIGEEDCAEFSGNGWDDKCNLAKFWICK
putative boDC-SIGN	-----
Chimp_DC-SIGN	KSAASCSRDEEQFLSPAPATPNPPPA--
huDC-SIGN_CDS	KSAASCSRDEEQFLSPAPATPNPPPA*
Macaca mulatta_CDS	KSAASCSGDEERLLSPAPTTPNPPPA*